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Review Article

A COMPREHENSIVE REVIEW ON ETHOSOMES

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ABSTRACT

Ethosomes are noninvasive delivery carriers that enable drugs to reach the deep skin layers and the systemic circulation. Although ethosomal systems are conceptually sophisticated, they are simple in their preparation, safe for use a combination that can highly expand their application. Ethosomes are soft, malleable vesicles tailored for enhanced delivery of active agents. Because of their unique structure, ethosomes are able to encapsulate and deliver through the skin highly lipophilic molecules such as cannabinoids, testosterone, and minoxidil, as well as cationic drugs such as propranolol, trihexaphenidyl, Cyclosporine, insulin, salbutamol etc. Enhanced delivery of bioactive molecules through the skin and cellular membranes by means of an ethosomal carrier opens numerous challenges and opportunities for the research and future development of novel improved therapies. Ethosomes are gaining popularity in designing drug delivery systems for topical and transdermal use for their capability to reach deep skin layers and systemic circulation. Although ethosomes are conceptually sophisticated, they are simple in preparation and safe for use. Although with their high efficiency, the ethosomes show potential for expansion of their applications. The aim of the review to make a comprehensive account on properties and preparation of ethosomes followed by the characterization and the list of drugs encapsulated in ethosomes in last 15 years.

Keywords: Ethosomes, malleable vesicles, ethosomal carrier, Transdermal

INTRODUCTION

Transdermal drug delivery is gaining importance due to its noninvasive procedure for administration. The transdermal drug delivery overcomes a number of limitations of oral drug delivery such as degradation of drugs by digestive enzymes, irritation of gastrointestinal mucosa and first pass effect. Also due the pain on administration associated with parenteral route, patients highly prefer transdermal route. Hence transdermal dosage forms enjoy being the most patient compliant mode of drug delivery. [1, 2]

Certain challenges to be addressed while designing Transdermal Dosage Forms

The skin is a multi-layered structure made up of stratum corneum (SC), the outermost layer, under which lies the epidermis and dermis. Within these layers of skin are interspersed fibroblasts, hair follicles and sweat glands that originate in the dermis blood supply. The almost unsurmountable nature of SC is a major challenge for systemic delivery of percutaneously applied drugs. [3] The Obrick and mortarO arrangement of corneocytes, flattened

mononucleated keratinocytes, with interspersed lipids and proteins makes the SC approximately 1000 times less permeable than other biological membranes. Furthermore, it is even more difficult for anything to penetrate to the deeper strata of skin. [4, 5]

Need for Transdermal Drug Delivery

Despite the challenges, TDD offers several unique advantages including relatively large and readily accessible surface area for absorption, ease of application and termination of therapy. Further, evolution of better technologies for delivering drug molecules, safe penetration enhancers and the use of vesicular carriers have rejuvenated the interest for designing TDD system for drugs that were thought to be unfit for transdermal delivery.

ETHOSOMES

The ethosomes are vesicular carrier consisting of hydroalcoholic or hydro/alcoholic/glycolic phospholipids in which the concentration of alcohols or their combination is relatively high. The ethosomes may contain phospholipids with various chemical structures like phosphatidylcholine (PC), hydrogenated PC, phosphatidic acid (PA), phosphatidylserine (PS), phosphatidylethanolamine (PE), phosphatidylglycerol (PPG), phosphatidylinositol (PI), hydrogenated PC, alcohol (ethanol or isopropyl alcohol), water and propylene glycol (or other glycols). [6] Such a composition enables delivery of high concentration of active ingredients through skin. Change in alcohol: water or alcohol-polyol: water ratio, alters drug delivery. The phospholipids generally used are soya phospholipids such as Phospholipon 90 (PL-90) in concentration range of 0.5-10% w/w. Cholesterol at concentrations ranging between 0.1-1% can also be used in the preparation to increase stability of ethosomes. Alcohols like ethanol and isopropyl alcohol and glycols like propylene glycol and Transcutol are generally used. In addition, non-ionic surfactants (PEG-alkyl ethers) in combination with the phospholipids are sometimes used in these preparations. Cationic lipids like cocoamide, POE alkyl amines, dodecylamine, cetrimide etc. can also be included. The concentration of alcohol in the final product may range from 20 to 50%. The concentration of the non-aqueous phase (alcohol and glycol combination) may range between 22 to 70%.

Table 1: Different Additives Employed In Formulation of Ethosomes. [6]

Class	Example	Uses
Phospholipid	Soya phosphatidyl choline	Vesicles forming component
	Egg phosphatidyl choline	
	Dipalmitylphosphatidyl choline	
	Distearylphosphatidyl choline	
Polyglycol	Propylene glycol	As a skin penetration enhancer
	Transcutol RTM	
Alcohol	Ethanol	For providing the softness for vesicle membrane
	Isopropyl alcohol	As a penetration enhancer
Cholesterol	Cholesterol	For providing the stability to vesicle membrane
Dye	Rhodamine-123	For characterization study
	Rhodamine red	
	Fluorescenelsothiocynate (FITC)	
	6- Carboxy fluorescence	
Vehicle	Carbopol D934	As a gel former

Advantage of high alcohol content

Ethanol is an established efficient permeation enhancer and is present in quite high concentration (20-50%) in ethosomes. However, due to the interdigitation effect of ethanol on lipid bilayers, it was commonly believed that vesicles could not coexist with high concentration of ethanol.

Touitou discovered and investigated lipid vesicular systems embodying ethanol in relatively high concentration and named them ethosomes. The basic difference between liposomes and ethosomes lies in their composition. The synergistic effect of combination of relatively high concentration of ethanol (20-50%) in vesicular form in ethosomes was suggested to be the main reason for their better skin permeation ability. The high concentration of ethanol (20-50%) in ethosomal formulation could disturb the

skin lipid bilayer organization. Therefore, when integrated into a vesicle membrane, it could give an ability to the vesicles to penetrate the SC. Furthermore, due to high ethanol concentration the ethosomal lipid membrane was packed less tightly than conventional vesicles but possessed equivalent stability. This allowed a softer and malleable structure giving more freedom and stability to its membrane, which could squeeze through small openings created in the disturbed SC lipids. In addition, the vesicular nature of ethosomal formulations could be modified by varying the ratio of components and chemical structure of the phospholipids. The versatility of ethosomes for systemic delivery is evident from the reports of enhanced delivery of quite a few drugs like acyclovir, minoxidil, triphexyphenidyl, testosterone, cannabidol and zidovudine. [12]

Methods of preparations of Ethosomes

Ethosomal formulation may be prepared by hot or cold method as described below. Both the methods are convenient, do not require any sophisticated equipment and are easy to scale up at industrial level.

1. Cold Method

In this method Phospholipids, drug and other lipid materials are dissolved in ethanol in a covered vessel at room temperature by vigorous stirring with the use of mixer. Propylene glycol or other polyol is added during stirring. This mixture is heated to 30^{0}C in a water bath. The water heated to 30^{0}C in a separate vessel is added to the mixture, which is then stirred for 5 min in a covered vessel. The vesicle size of ethosomal formulation can be decreased to the desire extent using probe sonication or extrusion method. Finally, the formulation is stored under refrigeration. [14]

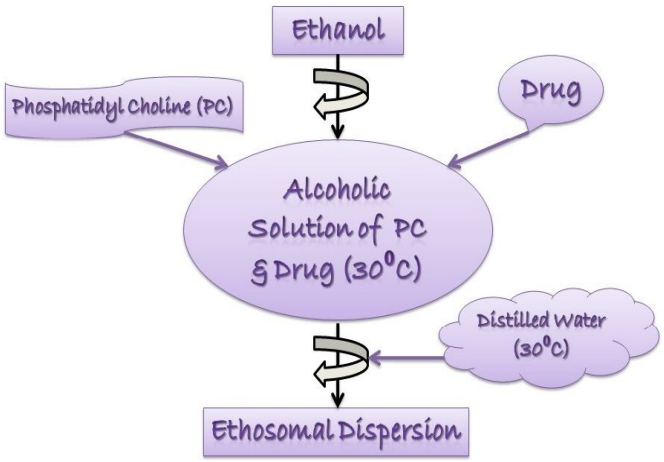


Fig.1: Preparation of Ethosomes by Cold Method

2. Hot Method:

In this method Phospholipid is dispersed in water by heating in a water bath at 40^{0}C until a colloidal solution is obtained. In a separate vessel ethanol and propylene glycol are mixed and heated to 40^{0}C. Once both mixtures reach 40^{0}C, the organic phase is added to the aqueous one. The drug is dissolved in water or ethanol depending on its hydrophilic or hydrophobic properties. The vesicle size of ethosomal formulation can be decreased to the desire extent using probe sonication or extrusion method. [14]

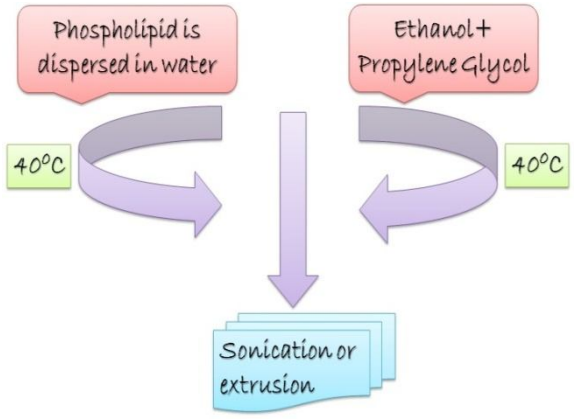


Fig.2: Preparation of Ethosomes by Hot Method

SKIN DELIVERY FROM ETHOSOMAL SYSTEM

The main advantage of ethosomes over liposomes is the increased permeation of the drug. The mechanism of the drug absorption from ethosomes is not clear. The drug absorption probably occurs in following two phases:

1. Ethanol effect
2. Ethosomes effect

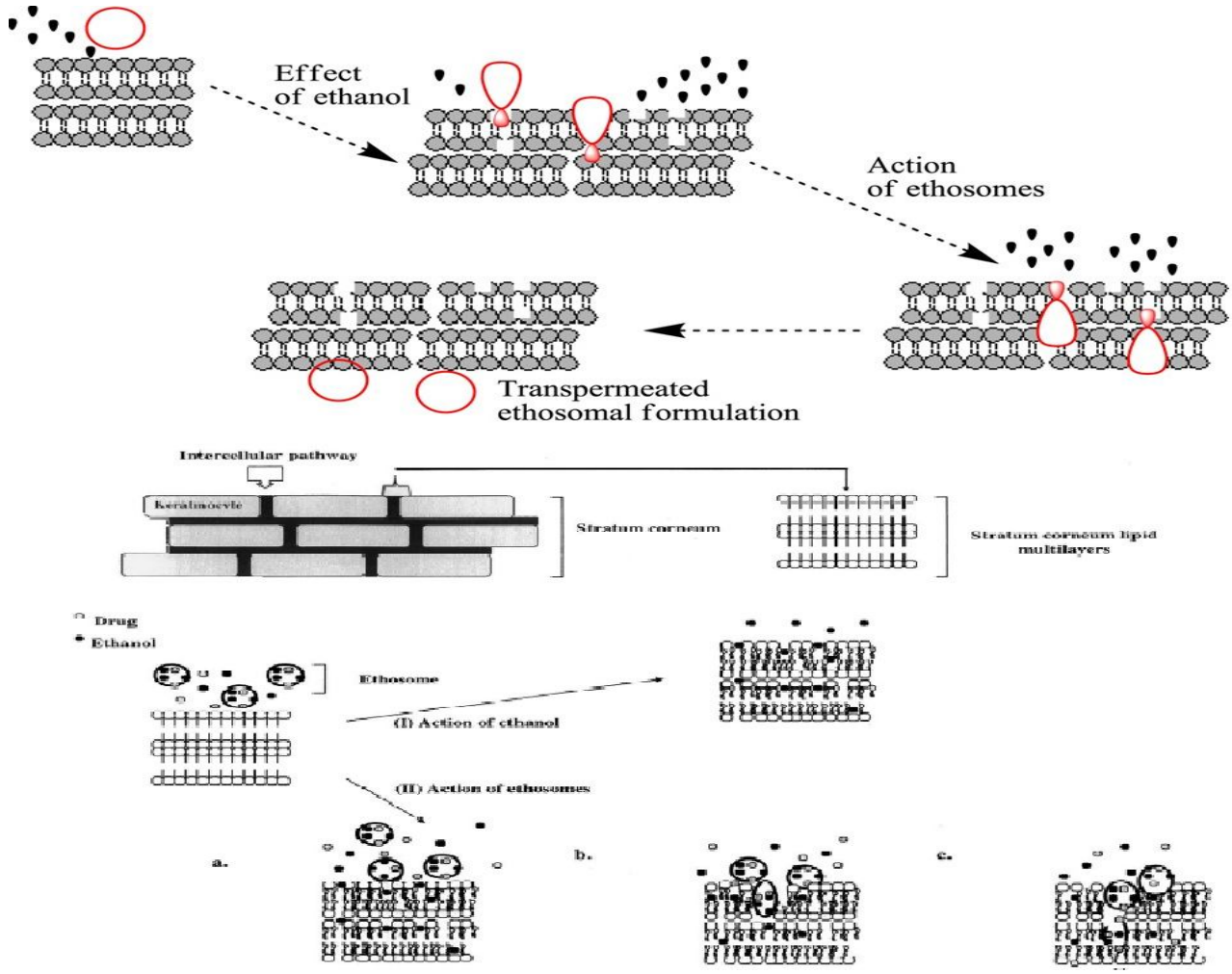
1. Ethanol effect

Ethanol acts as a penetration enhancer through the skin. The mechanism of its penetration enhancing effect is well known. Ethanol penetrates into intercellular lipids and increases the fluidity of cell membrane lipids and decrease the density of lipid multilayer of cell membrane.

2. Ethosomes effect

Increased cell membrane lipid fluidity caused by the ethanol of ethosomes results increased skin permeability. So the ethosomes permeates very easily inside the deep skin layers, where it got fused with skin lipids and releases the drugs into deep layer of skin. [12]

Fig.3: Mechanism of drug delivery from Ethosomes[12]



CHARACTERIZATION OF ETHOSOMES

Vesicle shape [2]

Transmission electron microscopy (TEM) and Scanning electronic microscopy (SEM) are used to characterize the surface morphology of the ethosomal vesicles. Prior to analysis, mount the ethosomes onto double sided tape that has previously been secured on copper stubs and coated with platinum, then analyzed at different magnifications.

Vesicle size and Zeta potential

Dynamic light scattering (DLS) using a computerized inspection system and photon correlation spectroscopy (PCS) are the two methods used in assessing the particle size and zeta potential of prepared Ethosomes.

Entrapment Efficiency [1]

Ultracentrifugation is the widely used technique to measure the entrapment efficiency of ethosomes. The vesicles are separated in a high speed cooling centrifuge at 20,000 rpm for 90 minutes in the temperature maintained at 4°C. Separate the sediment and supernatant liquids determine the amount of drug in the sediment by lysing the vesicles using methanol. From this, determine the entrapment efficiency by the following equation,

Entrapment efficiency = DE / DT x 100 Where,

DE - Amount of drug in the ethosomal sediment

DT - Theoretical amount of drug used to prepare the formulation (Equal to amount of drug in supernatant liquid and in the sediment)

Penetration and Permeation Studies [6]

Confocal laser scanning microscopy (CLSM) method is used to determine the depth of penetration from Ethosomes. The ethosomes shows significantly higher skin deposition possibly due to combined effect of ethanol and phospholipid thus providing a mode for dermal and transdermal delivery.

Transition Temperature [1]

The Transition temperature (T) of vesicular lipids is measured in duplicate by DSC in an aluminum pan at a heating rate of 10°C per min, under a constant nitrogen stream

Surface Tension Measurement [2]

Du Nouy ring tensiometer is used. Ring method is used to know the surface tension activity of drug in aqueous solution.

Vesicle Stability [2]

The ability of ethosomal preparations to retain the drug (i.e., drug-retentive behavior) can be checked by keeping the preparations at different temperatures, i.e., 25 ± 2°C (room temperature, RT), 37 ± 2°C and 45 ± 2°C for different periods of time (1, 20, 40, 60, 80 and 120 days). The ethosomal preparations were kept in sealed vials (10 ml capacity) after flushing with nitro-gen. The stability of ethosomes was also determined quantitatively by monitoring size and morphology of the vesicles using DLS and TEM.

Drug Content [1]

Drug can be quantified by a modified high performance liquid chromatographic method.

APPLICATIONS OF ETHOSOME [6]

Table 2: Application of Ethosomes as a Drug Carrier

Drug	Results
NSAIDS (Diclofenac)	✓ Selective delivery of drug to desired side for prolong period of time
Acyclovir	✓ Increase skin permeation ✓ Improved in biological activity two to three times ✓ Improved in Pharmacodynamic profile
Insulin	✓ Significant decrease in blood glucose level ✓ Provide control release
Trihexyphenidyl hydrochloride	✓ Improved transdermal flux ✓ Provide controlled release ✓ Improved patient compliance ✓ Biologically active at dose several times lower than the currently used formulation
DNA	✓ Better expression of genes ✓ Selective targeting to dermal cells
Antibiotic Cannabidol Erythromycin	✓ Improved skin deposition ✓ Improved biological activity ✓ Prolonging drug action
Bacitracin	✓ Improved dermal deposition ✓ Improved intracellular delivery ✓ Increased bioavailability ✓ Improved transdermal flux ✓ Improved in biological activity two to three times
Anti-HIV agents Zidovudine	✓ Improved in biological activity two to three times ✓ Improved in Pharmacodynamic profile

ETHOSOMES IN LAST 15 YEARS

Antihypertensive

E.g. Minoxidil [12]

NSAIDs

E.g. Aceclofenac [3], Diclofenac Potassium [16], Ketoprofen [17]

Antineoplastic agent

E.g. Methotrexate, Doxorubicin, Vincristine, bleomycin [18]

Antibiotics

E.g. Cannabidol [1], Erythromycin [19], Bacitracin [19]

Steroidal agents

E.g. Testosterone (Testosterone ®, Testoderm ®, Testosome ®) [20]

Beta blocker

E.g. Propranolol [21]

Antidiabetics

E.g. Insulin [22]

Antiasthmatics agents

E.g. Salbutamol [23]

Cerebrovascular agent

E.g. Ligustrazine [24]

Antiviral agents

E.g. Zidovudin [25], Lamivudine [26], Stavudine [27], Acyclovir [28, 29]

Antifungal agents

E.g. Clotrimazole [30]

Antiandrogen

E.g. Finasteride [31]

Herbal drugs

E.g. *Sophora alopecurides* (Alkaloids) [32], Fitoterapia [33], Tacrolimus [34], Paclitaxel [35],

ADVANTAGES OF ETHOSOMAL DRUG DELIVERY

1. Enhanced drug permeation through skin.
2. Delivery of large and diverse group of drugs (peptides, protein molecules).
3. Safe composition and the components are approved for pharmaceutical and cosmetic use.
4. Low risk profile.
5. High patient compliance.
6. Application in Pharmaceutical, Veterinary, Cosmetic field.

CONCLUSION

Ethosomes has initiated a new area in vesicular research for transdermal drug delivery. Ethosomes are characterized by simplicity in their preparation, safety and efficacy and can be tailored for enhanced skin permeation of active drugs. The main limiting factor of transdermal drug delivery system i.e. epidermal barrier can be overcome by ethosomes to significant extent. Ethosomes have been tested to encapsulate hydrophilic drugs, cationic drugs, proteins and peptides. Thus, ethosomal formulations possess promising future in effective transdermal delivery of bioactive agents.

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